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Resynchronization with the G6G protocol: a retrospective, observational study of second and later timed artificial inseminations on commercial dairy farms

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Sort running tittle: LONG BUT EFFICIENT RESYNCHRONIZATION PROTOCOL

Contents

We recorded conception rates and estimated pregnancy rates following second and later timed artificial inseminations (TAI) after hormonal resynchronization on commercial dairy farms, using the so–called G6G protocol (PGF day-0; GnRH 2, 8d; PGF 15, 16d, GnRH 17d; TAI 18d), and the 5 day Ovsynch protocol or 5DO (GnRH day 0; PGF 5, 6d; GnRH 7d; TAI 8d). In four farms both protocols were implemented in parallel, and these 1,368 second and later TAIs were used for the protocols' comparison based on logistic regression [544 TAIs in primiparous; 824 in multiparous cows; 1024 TAIs after G6G (600 TAIs in multiparous, 424 in primiparous); 344 TAIs after 5DO (224 TAIs in multiparous, 120 in primiparous); 280 TAIs during the hot season; 1088 during the cool season]. Conception rate (CR) was 31.7 ± 12.0% among all cows, 35.1 ± 10.7% among cows resynchronized with the G6G protocol and 21.8 ± 9.7% among cows resynchronized with the 5DO protocol (*P*<0.0001). CR among all cows was lower during the hot season (19.3 ± 8.4%) than during the cool season (34.9 ± 10.6%; *P*<0.0001), and similar seasonal results were observed with G6G protocols. Logistic regression showed significant effects on CR in second and later TAIs by protocol (OR=0.514; 95% CI 0.385 to 0.686; *P* < 0.0001) and season (OR=0.486; 95% CI 0.350 to 0.676; *P* < 0.0001). Parity did not influence CR after second and later TAIs (*P*>0.1) no interaction with season or resynchronization protocol was found. Estimated pregnancy rates based on these CR data from both hormonal protocols, suggest that G6G can be effectively used for second and later TAIs and highlight the importance of considering protocol and season when designing strategies for second and later timed AIs on dairy farms.

Keywords: **“**resynchronization”, “5dO” “season”, “parity”, “pregnancy rate”

1. INTRODUCTION

Decreasing cow fertility on dairy farms demonstrated in recent decades, to which a major contributing factor is ineffective estrous detection (Roelofs et al., 2010), has led many dairy farms to adopt the practice of timed artificial insemination (TAI) following synchronization of ovulation (Colazo and Mapletoft, 2014). Conception rates at the first TAI after parturition can be up to 50%, with success depending on several factors such as management practices, body condition, postpartum uterine diseases and hormonal treatments (Lopez-Helguera et al., 2012). Moreover, heat stress has been associated to a decreased fertility of herds (Garcia-Ispierto et al., 2007). To overcome these effects, several TAI systems have been proposed as hormonal therapy to mitigate decreased conception rates in hot season (Cartmill et al., 2001; Stevenson and Pulley 2012; review by De Rensis et al., 2015).

Strategies to maximize conception rates at the second and subsequent TAIs may substantially improve the reproductive rhythm of the herd and thereby overall reproductive efficiency. Several schemes have been developed to resynchronize cows after a negative pregnancy diagnosis (Forro et al., 2015) or even before their pregnancy status is known. For example, the original system of ovulation synchronization (Ovsynch protocol; Pursley et al., 1995) has been modified to shorten the interval between first administration of gonadotropin–releasing hormone (GnRH) and administration of prostaglandin from seven days to five (Santos et al., 2010; Colazo and Ambrose, 2015). This 5–day Ovsynch (5DO) protocol has been associated with conception rates of 45.3% (Colazo and Ambrose, 2015) and 34.7% (Motavalli et al., 2017), but it requires two doses of prostaglandin These rates and the short duration of the 5DO protocol make it an attractive option for resynchronizing cows, which should maximize pregnancy rates.

Much longer but also effective is the so–called G6G protocol, which involves presynchronization with prostaglandin, followed 2 days later with GnRH (100 mg) and then 6 days later with the first GnRH injection of the 7 d–Ovsynch procedure, corresponding to an interval of 18 days from first prostaglandin to TAI. A similar synchronization protocol was previously designed for first inseminations after calving (Stevenson and Pulley, 2012). G6G was also originally designed for first artificial inseminations (Bello et al., 2006), the G6G protocol reproducibly achieves high conception rates at the first TAI ranging from 35% (Astiz and Fargas, 2013) to 57% (Yousuf et al., 2016). This protocol causes a large proportion of cows being at the optimal stage of the cycle to initiate Ovsynch and improve most aspects of synchronization before or during the Ovsynch. The modification of introducing a second prostaglandin administration before TAI has been reported improving reproductive efficiency when compared to classical G6G (Heidari et al., 2017). Another variation with an initial prostaglandin 14d before initiation the G6G protocol increased progesterone concentrations before artificial insemination and decreased late embryonic/early fetal loss (Dirandeh et al., 2015). This modification enlarges in additional 14d the whole protocol, and makes this option not advisable for second and later TAIs.

A possibility that has yet to be investigated in detail is whether the G6G protocol can be used effectively and in an efficient way as a resynchronization protocol.

Few studies have directly observed resynchronization protocols by considering the risks of insemination and conception; multiplying these risks together gives the pregnancy rate, which is what dairy farms seek to maximize. Therefore, studies on resynchronization efficacy should examine all factors that affect either rate. These factors include the days until first pregnancy diagnosis, which influences the interval between inseminations (Silva et al., 2009). Another important factor is parity, which has been associated with fertility (De Kruif, 1978) and with conception rate within synchronization protocols (Astiz and Fargas, 2013; Marques Mde et al., 2015). Finally heat stress can decrease fertility (De Rensis and Scaramuzzi, 2003) and reduce uterine blood flow as well as oocyte quality, reducing the effectiveness of insemination (Roth et al., 2001). Many of these negative factors could be overcome, in part, by employing hormonal strategies.

Our hypothesis was that G6G, although even being a presynchronization protocol initially thought for first inseminations, and much longer than any other resynch protocol, can be as efficient as a short one (5DO) for second and later TAIs if the achieved conception rate is higher enough than that obtained after the 5DO protocol, to counteract the advantageous shorter length of the 5DO protocol.

Therefore, the aim of the present study was to describe conception rates and estimated pregnancy rates after a scarcely used for resynchronization protocol (G6G) and 5DO resynchronization for second and later TAIs in cows, on commercial dairy farms. The analysis on the retrospective data took into account factors suspected to influence conception and pregnancy rates: parity, season of TAI, hormone brands and time until first pregnancy diagnosis.

2. MATERIAL AND METHODS

2.1. Animals and Experimental Procedures

This study included retrospective farm data for 4,279 second and later TAIs in dairy Holstein cows (2,809 TAIs in primiparous cows, 1,470 in multiparous cows) from 29 high–yield commercial farms in eastern Spain.

The mean number of cows in milk was 400 per farm, and the mean milk yield per cow per lactation was 10,507 L. All herds were managed under similar conditions, which included *ad libitum* access to water and twice–daily feeding with a total mixed ration (TMR) that was balanced to meet or exceed nutrient recommendations for lactating dairy cows (NRC 2001).

One first part of the analysis aimed to compare Conception Rates (CR) from second and later inseminations after two hormonal resynchronization protocols implemented in parallel in four farms. The G6G resynchronization protocol (Figure 1; Bello et al., 2006) was used with the modification of two prostaglandin doses before insemination (Heidari et al., 2017; Wiltbank et al., 2015). The protocol consisted of prostaglandin administration on day 0, GnRH on days 2 and 8, prostaglandin on days 15 and 16, GnRH on day 17 (afternoon), and finally TAI on day 18 (Figure 1). This meant an interval of 18 days between protocol start and TAI. The 5DO protocol (Bisinotto et al., 2010) consisted of GnRH on day 0, two prostaglandin doses on days 5 and 6, GnRH on day 7 (afternoon), and finally TAI on day 8 (morning; Figure 1). This meant an interval of 8 days between protocol start and TAI.

The GnRH analogues used were gonadorelin diacetate 100 µg (Cystoreline; Ceva SA, Barcelona, Spain) or buserelin acetate 0.0042 mg (Receptal; MSD, Boxmeer, Netherlands). The following analogues of prostaglandin $F_{2\alpha}$ (PGF) were used: cloprostenol sodium 500 µg (Cyclix; Virbac SA, Barcelona, Spain) and dinoprost tromethamine 5 mg/ml (Dinolytic, Pfizer, Paris, France; or Enzaprost, Ceva SA, Barcelona, Spain). The choice of analogues was made arbitrarily by the farms themselves, reflecting the retrospective nature of our study. The doses used were in all cases those doses recommended by the drug producer. The use of the different hormones was also included into the model, as fixed effect.

Cows were not subjected to ultrasound examinations to assure the absence of anestrus or ovarian diseases, prior to inclusion in the resynchronization protocol. Pregnancy diagnoses were performed by transrectal palpation or ultrasound between 28 d and 45 d after TAI, without recording ovarian status, and the resynchronization protocol was started on the day of pregnancy diagnosis ±three days in all non–pregnant cows. However, actual interinsemination intervals were not available. At these farms where both protocols were used in parallel the weekly basis was preferred. However, this distribution was not consistently followed, which explains the unbalanced amount of TAIs (more G6G TAIs than 5DO TAIs). In these farms four inseminators (one per farm) randomly performed all AIs. TAIs performed in the other 25 farms were made by farm inseminators. No synchronization rate data were available, and only second and later TAIs after finished protocols were included.

This study included 1,368 second and later TAIs (544 TAIs in primiparous cows, 824 in multiparous cows). A total of 1,024 second and later TAIs were recorded in animals resynchronized using G6G (600 TAIs in multiparous cows, 424 in primiparous cows). A total of 344 second and later TAIs were performed in animals resynchronized using 5DO (224 TAIs in multiparous cows, 120 in primiparous cows). Finally, a total of 280 second and later TAIs were performed during the hot season and 1,088 during the cool season. The included TAIs depending on farm were Farm-1 with 519 TAIs (47 after 5dO and 472 after G6G); Farm-2 with 648 TAIs (163 after 5dO and 485 after G6G); Farm-3 with 31 TAIs (5 after 5dO and 26 after G6G); and Farm-4 with 170 TAIs (129 after 5dO and 41 after G6G).

The second subset of second and later TAIs (n=2,098) came from a total of 25 farms that only implemented one of the two protocols. This data were used to describe farm average Conception Rate (CR) results of those farms, without statistical comparisons among protocols (treatment was in this case cofounded with farm). G6G was exclusively implemented on 19 farms and 5DO on 6 farms.

All activities within the study were routine farm practices, with no ethical approval required following the European Union Directive 2010/63/UE.

2.2. Outcomes and Variables

Data were collected on parity (primiparous *vs.* multiparous) and season at TAI, with the hot season defined as June, September, and October; and the cool season defined as November–May. No resynchronization protocols were carried out in July or August since temperatures during these months are usually too high and pregnancy rates too low to make synchronizations profitable. Interactions between parity and season as well as interactions among these factors and resynchronization protocol were also analyzed.

2.3. Statistical Analyses

Data were analyzed using SPSS® 22.0 (IBM, USA) to identify relationships among synchronization protocol, parity and season. The experimental unit was the TAI in the subset of TAIs performed in the four farms, where both protocols were implemented in parallel. The statistical model including fixed effects of two resynchronization protocols (G6G *vs.* 5dO), season, parity, hormone brand and all interactions were applied, with farm as random variable. Binary data were analyzed by logistic regression using a stepwise forward method based on the Wald statistic criterion of $P > 0.10$, to obtain the odds ratio values (OR) with associated 95% confidence interval (95%CI).

All data are reported as mean ± SD and, where appropriate, as an odds ratio (OR) with associated 95% confidence interval (95%CI).

Data referred to the other subset of TAIs were described as farm average Conception Rates and was considered relevant as example of farm extensive use of these hormonal protocols as resynchronization protocols.

3. RESULTS

3.1. Comparison between reproductive results after G6G *vs.* 5DO hormonal protocols use for second and later TAIs.

Mean conception rate was 31.7 ± 12.0% among all cows undergoing second and later TAIs, 35.1 ± 10.7% among cows resynchronized using the G6G protocol and 21.8 ± 9.7% among cows resynchronized using the 5DO protocol (Table 1). Parity did not significantly affect conception rate: it was 31.7 ± 11.7% among primiparous cows and 32.1 ± 12.2% among multiparous ones (P = 0.098).

Farm CR (%) was significantly different among farms (P < 0.0001) when analyzed alone, with Farm-1 CR being 39.5±10.4%; Farm-2 25.8±6.2%; Farm-3 with 42.0±28.8% and Farm-4 28.8±13.6%, but in the logistic regression model, when taking into account all other fixed effects, farm did not result significantly affecting CR values.

Logistic regression showed significant effects on Conception Rate in second and later TAIs by Synchronization Protocol (21.8±9.7 vs. 35.1 ±10.7% for 5dO and G6G, respectively; OR=0.514; 95% CI 0.385 to 0.686; P < 0.0001) and season (19.3±8.4 vs. 34.9 ±10.6% for the hot and cool season, respectively; OR=0.486; 95% CI 0.350 to 0.676; P < 0.0001). The factors “farm”, “parity”, “hormone brand” and any interaction among the factors did not influence the CR in the statistical model (P>0.1).

3.2. Descriptive reproductive data on second and later TAIs from 25 dairy cattle farms using G6G or 5DO hormonal protocols for resynchronization.

Average farm conception rates including second and later TAIs performed during the all year ranged from 0.0 to 73.1% for all the cows and from 0 to 67% and 0 to 100% when divided in multi– and primiparous subgroups, respectively. Mean farm conception rate was 38.2 ± 14.6% among all cows undergoing second and later TAIs, 28.2 ± 14.8% during the hot season and 40.9 ± 15.0% during the cool season. CR average values during the different seasons and depending on parity are resumed in Table 2.

3.3. Estimated pregnancy rates

In order to estimate the theoretical reproductive efficiency (i-e- estimated maximal pregnancy risk or PR) based on the observed CR after each resynchronization protocol analyzed in this study, CR values were multiplied by insemination rates, during each season as a function of when pregnancy was diagnosed after TAI (Table 3).

4. DISCUSSION

This study suggests that season and resynchronization protocol, but not parity, affect conception rate during the second and later TAI in dairy commercial farms. Our results also suggest that the G6G resynchronization protocol, although long, may provide satisfactory conception and estimated pregnancy rates.

Productive farms aim to get the most cows pregnant in the shortest period of time following the voluntary waiting time (Poock 2009). This leads many farms to select shorter resynchronization protocols in order to intensify the reproductive rhythm (Ribeiro, 2012; Giordano et al., 2013b). Our data suggest, and the theoretical figures recorded in table 3, however, that a longer protocol can be as effective as a shorter one if the conception rate is higher enough to overcome longer interinsemination intervals. At the same time, the interval between TAI and pregnancy diagnosis is important: the later that pregnancy is diagnosed, the more the estimated pregnancy rate with either protocol is influenced by the expected conception rate (Table 3), and less by the interval between inseminations. Although actual interinsemination intervals were not available, our results confirm previous studies demonstrating the importance of the interval between TAI and pregnancy diagnosis (Sinedino et al., 2014; Giordano et al., 2015; WIjma et al., 2017).

The average conception rate observed after the 5DO protocol in our study is lower than the 34.7% or 45.3 reported by other authors in the absence of heat stress (Colazo and Ambrose, 2015; Motavalli et al., 2017). This discrepancy may be due to the heat stress factor, always present in Spain, where all the farms were placed. However, we have to highlight the large variation range of CRs across the farms in our study, which can make that these lower rates were not real.

Mean conception rate after the longer presynchronization protocol such as G6G is expected to be over 30%, as found in our study. Subluteal progesterone (P4) concentrations worsen ovulatory follicle development and subsequent fertility in cattle (Revah and Butler, 1996). On the other hand, high P4 concentrations at the AI due to inadequate CL regression may interfere with semen transport and fertilization (Graham and Clarke, 1997) and also impair the normal ovulation process (Bridges and Fortune, 2003) resulting in reduced fertility (Giordano et al., 2012b; Lopes et al., 2013; Behrouzi et al., 2016). The inclusion of a presynchronization of the estrous cycle before TAI and the application of two doses of prostaglandin before TAI can solve in some extent those problems. The presynchronization step in the G6G protocol induces optimal conditions for the Ovsynch injections (Sterry et al., 2006). This presynchronization may have been particularly beneficial in the present study, which included cows that failed to become pregnant after one previous artificial insemination, regardless of the cycle stage of the ovaries. Another explanation is that protocols such as G6G that optimize the physiological milieu in the uterus before TAI can overcome problems of anovularity or physiological limitations that preclude cycling (Bello et al., 2006; Bisinotto et al., 2010; Dewey et al., 2010; Giordano et al., 2013a). Therefore, a better CR after G6G protocol than after 5DO was expected. Moreover, recent studies proposed a differentiated resynchronization strategy, based on the ovarian status, i.e. the presence of a corpus luteum ≥15 mm and a follicle ≥10 mm at the time of pregnancy diagnosis. The new strategy did not enhance conception rate but reduced time to pregnancy because of a reduction of the TAI-TAI interval for cows with a CL at pregnancy diagnosis (WIjma et al., 2017). This study highlights again the relevance of the interval TAI-TAI when deciding resynchronization programs in dairy cows.

In our study, the conception rate was significantly lower in the hot season (19.3± 8.4%) than in the cool season (34.9 ± 10.6%; $P < 0.0001$) independently from parity and synchronization protocol. Similar results have been reported by others (De

Rensis and Scaramuzzi, 2003), including in cows undergoing a second and later insemination (Giordano et al., 2012b). Multiple physiological mechanisms are likely to explain this relationship between conception rate and season (Hansen and Arechiga, 1999). In general, it is complicated to overcome the several physical effects triggered by the heat stress on the ovarian and uterine structures exclusively with hormonal strategies. Several TAI strategoes have been proposed as hormonal therapy to mitigate decreased conception rates in hot season (reviewed by De Rensis et al., 2015; Dirandeh et al, 2015; Voelz et al., 2016), For example, the practice of administering GnRH repeatedly in the autumn (after the hot season) in order to accelerate follicular turnover and thereby enhance fertility has been described (Roth et al., 2004; Friedman et al., 2014). However, other strategies have failed to improve fertility in heat stress cows (Patron-Collantes et al., 2017). In our study we could neither detect any statistically significant interaction between protocol and season, suggesting any positive effect of the hormonal protocols included in this study on the CR of second and more TAIs.

We did not observe any effect of parity on conception rate during the second and later TAIs, in contrast to numerous studies showing that primiparous cows are more fertile than multiparous ones at the first insemination (Lima et al., 2012), and in contrast to at least one study showing greater fertility in primiparous cows than multiparous ones at second and later inseminations (Sterry et al., 2006). Our study is not alone in this regard: other studies have observed similar conception rates between primiparous and multiparous cows after resynchronization and TAI (Dewey et al., 2010; Forro et al., 2015). This has even been observed with the Double Ovsynch resynchronization protocol (Giordano et al., 2013a), even though this protocol, when used at the first insemination, leads to a notably higher conception rate in primiparous cows than in multiparous cows (Souza et al., 2008; Astiz and Fargas, 2013). The relationship between parity and fertility is poorly understood and likely to be complex. For example, one study has reported higher fertility in cows with three or more lactations than in cows with one or two (Alkar et al., 2011). Parity has different effects on fertility. According to El-Razek et al. (2017) the conception rate decreases in multiparous cows, after being synchronized with G6G coinciding with Yusuf et al (2017) and the studies of LeBlanc et al (2002), in which it is suggested that the decrease of the CR is due to the higher risk in multiparous cows of suffering clinical endometritis. Yusuf et al. (2010) also suggest that cows with more than 4 births have more DeVries et al. (2011) adds the role of the Negative Energy Balance, which affects more intensively multiparous than primiparous cows. In turn, it has been observed that the increase of parity causes a significant enlargement in days open (Elmetwally et al., 2016), but not in the return to ovarian activity after calving.

However, not many studies focus on the difference in CR associated to parity when only second and later TAIs re observed (Dewey et al., 2010; Forro et al., 2015). One explanation for a lack of effect of parity on conception rate specifically at second and later TAIs is that primiparous cows have lost certain advantages of reproductive physiology from not having produced milk before. One study reporting similar conception rates between primiparous and multiparous cows (Dewey et al., 2010) found smaller dominant follicles and less luteal tissue in resynchronized cycles of primiparous cows than in multiparous cows. Reduced levels of progesterone in the circulation prior to artificial insemination have been linked to lower fertility in lactating dairy cows (Bisinotto et al., 2015), and the extent of progesterone reduction correlates with high milk yield and daily dry matter intake (Sartori et al., 2004).

Primiparous cows produce less total and peak milk than multiparous cows but have greater lactational persistency (Lean et al., 1989), and they may show a more negative energy balance. This leads us to hypothesize that after primiparous cows have produced high levels of milk for a certain time – which was the case of the cows in our study – their reproductive physiology matches that of their multiparous counterparts, resulting in similar conception rates. Further studies are required to explore this hypothesis.

A strength of the present study is that it describes a scarcely used resynchronization protocol (G6G) in a large number of inseminations across commercial dairy farms, making it more likely to indicate actual efficacy, independent of farm effects and a prolonged interinsemination interval. Moreover it detects a lack of effect the factor parity at these second or later TAIs. Limiting the ability of our results to guide protocol selection is the retrospective, observational design of the study. As a result, we could not control for other factors that influence conception rate after resynchronization, such as body condition score (Forro et al., 2015), somatic cell count, number of inseminations or days in milk at insemination (Giordano et al., 2015). Finally, we did not record early pregnancy loss rates, which can heavily influence decisions to use one or another resynchronization protocol on farms (Giordano et al., 2012a).

Despite these disadvantages, the large number of TAIs in this study give it sufficient statistical power to derive robust conclusions about resynchronization, season, parity and their interactions in the absence of local, farm–specific confounding factors. In this sense, the pregnancy rates that we estimated based on timing of first pregnancy diagnosis, protocol and season may be a useful "rough guide" for farms when choosing a resynchronization protocol based on their own records.

CONCLUSIONS

With this study, a rarely used "G6G" hormonal protocol is demonstrated to effectively resynchronize herds and increase the options available for artificial insemination. We also provide evidence that hormonal protocol and season but not parity, should be considered when designing strategies for second and later timed artificial inseminations.

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CONFLICT OF INTEREST STATEMENT

Authors has no conflict to declare.

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AUTHORS CONTRIBUTIONS

RP and AS designed data collecting and study design. RP, ILH and JL PP collected data. JH, JVG, OF and SA processed farm and global data. RP, NPV, OF and SA wrote manuscript and all authors revised it until its final version.

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Table 1

Conception rates of second and later TAIs in primiparous and multiparous cows after G6G or 5DO resynchronization, stratified by season of insemination.

	Resynch	All year		Hot season		Cool season		
	Protocol	TAI, n	% CR	TAI, n	% CR	TAI, n	% CR	*P* value†
Herd	5DO	344	21.8 ± 9.7	65	20.0 ± 14.8	279	22.2 ± 8.1	= 0.098
	G6G	1024	35.1 ± 10.7	215	19.1 ± 5.1	809	39.3 ± 7.3	< 0.0001
	Total	1368	31.7 ± 12.0	280	19.3 ± 8.4	1088	34.9 ± 10.6	< 0.0001
Prim	5DO	120	23.3 ± 14.4	17	23.5 ± 24.3	103	23.3 ± 12.3	= 0.952
	G6G	424	33.3 ± 9.7	115	20.0 ± 1.7	309	38.1 ± 6.3	< 0.0001
	Total	544	31.1 ± 11.7	132	20.5 ± 8.7	412	34.4 ± 10.4	< 0.0001
Mult	5DO	224	20.1 ± 5.7	48	18.8 ± 9.6	176	21.6 ± 3.9	= 0.002
	G6G	600	36.3 ± 11.2	100	18.0 ± 7.2	500	40.0 ± 7.8	< 0.0001
	Total	824	32.1 ± 12.2	148	18.2 ± 8.0	676	35.2 ± 10.7	< 0.0001

See Methods for detailed descriptions of the 5DO and G6G resynchronization protocols. Conception rates are reported as percentages (mean ± SD) of pregnancies per timed artificial insemination.

Abbreviations: Herd, all animals; Mult, multiparous cows; Prim, primiparous cows; TAI, timed artificial insemination.

† For comparisons of conception rates during the hot or cool season.

Table 2

Farm Conception rates of second and later TAIs in primiparous and multiparous cows after G6G or 5DO resynchronization, stratified by season of insemination. †

	Resynch Protocol	All year Farms, n	All year % CR	Hot season Farms, n	Hot season % CR	Cool season Farms, n	Cool season % CR	*P* value‡
Herd	5DO	6	31.1 ± 4.9	3	27.8 ± 16.8	6	32.6 ± 4.0	0.713
	G6G	19	40.4 ± 16.3	13	28.5 ± 15.6	19	43.3 ± 16.7	0.04
	Total	25	38.2 ± 14.6	16	28.2 ± 14.8	25	40.9 ± 15.0	0.038
Prim	5DO	5	24.6 ± 15.6	3	29.2 ± 26.0	5	25.4 ± 16.0	0.940
	G6G	18	45.7 ± 24.8	12	31.0 ± 25.4	18	48.2 ± 24.7	0.163
	Total	23	41.0 ± 23.9	15	30.4 ± 23.8	23	43.3 ± 24.2	0.282
Mult	5DO	6	37.5 ± 15.5	3	26.5 ± 7.7	6	39.8 ± 15.3	0.438
	G6G	19	37.5 ± 12.7	13	27.1 ± 10.9	19	41.0 ± 14.2	0.014
	Total	25	37.5 ± 12.8	16	26.8 ± 9.8	25	40.8 ± 13.9	0.005

† Due to the non-experimental design, and because each farm implemented a different synchronization protocol and treatment is confounded with farm no comparison is made between CR between protocols.

See Methods for detailed descriptions of the 5DO and G6G resynchronization protocols. Conception rates are reported as percentages (mean ± SD) of pregnancies per timed artificial insemination.

Abbreviations: Herd, all animals; Mult, multiparous cows; Prim, primiparous cows; TAI, timed artificial insemination.

‡ For comparisons of conception rates during the hot or cool season.

Table 3

Estimated, theoretical pregnancy rates in cows resynchronized with 5DO or G6G protocols, stratified by season of timed artificial insemination and by number of days after TAI that pregnancy was diagnosed.

Protocol	Days at PD	Length (d)	TAI– TAI (d)	IR	All year		Hot season		Cool season	
					CR	PR	CR	PR	CR	PR
5DO	27	8	35	60%	22%	13,20%	20%	12,00%	22%	13,20%
G6G	27	18	45	47%	35%	16,45%	20%	9,40%	39%	18,33%
5DO	31	8	39	54%	22%	11,88%	20%	10,80%	22%	11,88%
G6G	31	18	49	43%	35%	15,05%	20%	8,60%	39%	16,77%
5DO	34	8	42	50%	22%	11,00%	20%	10,00%	22%	11,00%
G6G	34	18	52	40%	35%	14,00%	20%	8,00%	39%	15,60%
5DO	38	8	46	46%	22%	10,12%	20%	9,20%	22%	10,12%
G6G	38	18	56	38%	35%	13,30%	20%	7,60%	39%	14,82%
5DO	41	8	49	43%	22%	9,46%	20%	8,60%	22%	9,46%
G6G	41	18	59	36%	35%	12,60%	20%	7,20%	39%	14,04%

See Methods for detailed descriptions of the 5DO and G6G resynchronization protocols.

Days at PD, number of days between TAI and pregnancy diagnosis; Length (d), number of days between the start of the resynchronization protocol and subsequent TAI; TAI– TAI, minimal interval in days between TAIs; IR, insemination rate (21 days/TAI–TAI); CR, conception rate (pregnancies per TAI); PR, estimated pregnancy rate; TAI, timed artificial insemination

Figure legends

Figure 1. Schedule of hormonal treatments in lactating dairy cows according to the 5DO (5 days Ovsynch) and G6G resynchronization protocols, ending with second and later TAI. GnRH, gonadotropin–releasing hormone; PGF, prostaglandin $F_{2\alpha}$; TAI, timed artificial insemination. Non-pregnancy diagnosis was performed 0 to 3 days before beginning of the hormonal protocols.